

Original Research Article

Isolation, characterization and evaluation of antioxidant activities of secondary metabolites producing actinomycetes of terrestrial origin

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ABSTRACT

Background: Microbial secondary metabolites are important sources of natural compounds when compared to others with potential, beneficial therapeutic applications. There are chances of discovery of new *Streptomyces* species and new compounds from the respective genus. Due to ever and over increasing resistance of pathogenic bacteria to our current arsenal of antibiotics, a great need exists for the isolation and discovery of new antibiotics and other drug agents. Based on the above concept actinomycetes are mainly targeted for secondary metabolites production and evaluation of compounds therapeutically.

Methods: Totally 15 pure isolates were obtained from two different soil samples by spread plate and quadrant streak techniques. Their colony and surface morphology were studied by morphological and biochemical characterization. Secondary metabolites were extracted by solvent extraction and the presence of bioactive compounds was detected by thin layer chromatography. The antioxidant potential was determined by Dot plot, DPPH and Phosphomolybdenum assay. The nature and the number of active compounds were identified by GC-MS analysis.

Results: Among the 15 isolates, 10 isolates were found to have characteristic features of actinomycetes and 8 isolates were found to be fast growing actinomycetes. Among these 8 isolates, isolates of terrestrial origin were found to possess more bioactive compounds than those of marine origin and the four terrestrial isolates selected for evaluation of bioactive compounds and determination of antioxidant potential showed that the isolate TS 1010 had better and efficient secondary metabolite activity.

Conclusions: Thus, from the above study it is seen that not only actinomycetes of marine origin, but terrestrial origin are also fast growers and possess better antioxidant and radical scavenging activity.

Keywords: Actinomycetes, Dot plot assay, DPPH assay, GC-MS, Phosphomolybdenum assay, TLC

INTRODUCTION

The sea, covering more than 70% of the surface of planet Earth, contains an exceptional biological diversity,

accounting for more than 95% of the whole biosphere.¹ Microbial diversity constitutes an infinite pool of novel chemistry, making up a valuable source for innovative biotechnology.^{2,3} Actinomycetes have a profound role in

the marine environment apart from antibiotic production. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms. Actinobacteria, which are the prolific producers of antibiotics and important suppliers to the pharmaceutical industry, can produce a wide variety of secondary metabolites.⁴ Representative genera of actinobacteria include *Streptomyces*, *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Micromonospora* and several others. Secondary metabolites produced by the marine actinobacteria possess a wide range of biological activities.^{2,5-8} TLC is generally regarded as a simple, rapid, and inexpensive method for the separation, tentative identification, and visual semi quantitative assessment of a wide variety of substances. Thin layer chromatography is used to separate components in a mixture and to identify unknown materials by comparing their separation pattern to that of known reference materials.⁹

Antioxidants (AA) slow down the process of degradation so that the energetic action of the environment can lead to higher sustainability. They interact with FR, making possible their reaction with oxygen. Antioxidants can be grouped into two classes-synthesis antioxidants and natural antioxidants. The difference between the two categories is that most synthesis antioxidants generate substances that develop cancer or other diseases. Classifying antioxidants can be done depending on their function or on their nature. Depending on their function, there are: Primary antioxidants: ascorbic acid and its derivatives, tocopherols, the esters of gallic acid, erythorbic acid and its sodium salt, BHA, BHT and other substances THBP and TBHQ. Secondary antioxidants (substances with antioxidant action but that have other functions as well). Sulphur dioxide and sulphites as well as lecithin are secondary antioxidants. Besides the above-mentioned compounds, there are also substances that can strengthen the action of primary antioxidants such compounds as lactic acid and lactates, citric acid and citrates, tartaric acid and tartrates, etc. have either a synergetic or action of metal complexes that catalysis self-oxidation reactions.

Depending on their nature, AA can be natural or synthesis. Tocopherols (TCP) are natural AA, as well as a series of compounds with different structures that can be found in plants (flavones). The most important class of synthesis AA act by interrupting the lipid self-oxidation reaction chain.¹⁰ Free Radicals are molecules with an unpaired electron and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission. Radiolysis is a powerful method to generate specific free radicals and measure their reactivity. Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability. A chain reaction thus gets started. Once the process is started, it can cascade, and finally results in the disruption of a living cell. Free radicals cause many human diseases like

cancer Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease, fibrosis, etc. The free radicals formed in our body are combated by antioxidants that safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

Antioxidants are intimately involved in the prevention of cellular damage the common pathway for cancer, aging, and a variety of diseases. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-"stealing" reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease. Vitamin E is the most abundant fat-soluble antioxidant in the body. It is one of the most efficient chain-breaking antioxidants available, is the primary defender against oxidation, and is the primary defender against lipid per oxidation (creation of unstable molecules containing more oxygen than is usual). Vitamin C - The most abundant water-soluble antioxidant in the body. It acts primarily in cellular fluid. It combats free-radical formation caused by pollution and cigarette smoke. Also helps return vitamin E to its active form.¹¹ The main aim is to screen the isolated actinomycetes collected from Chennai, Tamil Nadu, for the evaluation of the bioactive compounds producing actinomycetes with effective radical scavenging activity.

METHODS

Soil sample collection

A total of two soil samples, were collected from rhizosphere regions of *Manilkara zapota* (sapota) and inner depths of muthukadu region.

The soil samples were collected from a depth of 15 cm in sterile plastic bags during December 2016. Then they were air-dried at room temperature for a period of five days and processed with standard microbiological methods.

Isolation of Actinomycetes¹⁷

Starch casein agar (SCA) medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min. Then it was supplemented with fluconazole (30µg/l) to prevent the bacterial and fungal growth.¹⁰

The medium was poured into the sterile Petri plates. The collected soil samples were diluted from 10-1 to 10-10 and 0.1 ml of the diluted samples was spread over the agar medium. The inoculated plates were incubated at 28±2°C for 7 to 10 days.

After incubation, the actinomycetial colonies were purified by streak plate technique, sub-cultured and stored at 4°C on SCA slants for further investigation.

Preliminary characterization of Actinomycetes isolates

Colony morphology¹⁸

Colony morphology of the purified actinomycetial isolates on SCA medium were recorded with respect to colour of aerial spore mass, size and nature of the colonies, colour on the reverse side and diffusible pigmentation.

Light microscopy (coverslip culture technique)¹⁹

Starch casein agar (SCA) medium was prepared and 3 to 4 sterile coverslips were inserted at an angle of 45°. The purified actinomycetial culture plates were carefully streaked on the surface of coverslip without any damage. The plates were incubated at 28±2°C for 4-8 days. The coverslips were removed at 2-3 days of interval and observed under the high-power magnification. The structure and arrangement of conidiospores and arthospores on aerial and substrate mycelia were observed and compared with Bergey's Manual of Determinative Bacteriology.

Extraction of bioactive compounds from selected Actinomycetes

The isolates were inoculated in International Streptomyces Project (ISP-2) medium and kept at 37°C for 10 days at 150rpm. After incubation the broth was filtered through Whatman No.1 filter paper, the culture filtrate was centrifuged at 8,000 rpm for 15 min. Equal volume of ethyl acetate was added at 1:1 ratio. The mixture was kept undisturbed overnight for complete production. The organic solvent extract were evaporated to dryness, dissolved in specific solvents and subjected to antioxidant studies and further analytical methods.¹¹

Biochemical characterization²⁰

Gram staining

A loopful of culture was placed in a clean glass slide and it was heat fixed by gently passing over the flame. The smear was flooded with crystal violet and allowed to stand for one minute.

The slide was then washed with tap water. Again the smear was flooded with gram's iodine mordant and was allowed to stand for one minute. The slide was washed with tap water.

The slide was decolorized using 95% ethyl alcohol. The decolourizer was added drop by drop until crystal violet gets drained out from smear. The slide was washed again with tap water and it was counter stained with safranin for 45seconds. Then, the slide was washed with tap water. The slide was air dried and examined under microscope.

Diffusible pigment production test

The actinomycetes isolates were inoculated into Glycerol Asparagine agar medium and incubated at 28±2°C for 14 days. The formation of colour such as yellow- brown, blue, green, red, orange and grey or violet in complete medium was recorded.

Melanin pigment production test

Melanin pigment production was considered to cause browning of organic media namely tyrosine agar medium containing tyrosine. The tyrosine agar slants were inoculated with actinomycetes isolates and incubated at 28±2°C. After 2-4 days, production of soluble pigments, colours of vegetative and aerial mycelium in the slants were observed.

Dot-blot assay for rapid radical scavenging activity for the crude extracts

To the pre-coated TLC plate (silica gel 60 F254; Merck), drops of DPPH solution dissolved in methanol were loaded onto each column accordingly. The TLC plates were allowed to dry for 3-5 minutes.

DPPH solution was stained in the first row of TLC plate and was considered as control. The crude compound (various samples) in various concentrations was carefully loaded onto the DPPH stained spot in the respective rows. The next row of TLC plate was considered as the standard reference, where ascorbic acid was carefully loaded onto the DPPH stained spot.

The staining of the silica plate was based on the procedure of Soler- Rivas et al.⁹ Purple colour with yellow or white spots on the TLC plates where radical scavenging capability observed was exhibited on the stained silica gel plates. The vanishing effect of purple colour depends upon the amount and nature of antioxidants (active compounds) present in the extracted secondary metabolites.

Thin layer chromatographic analysis of bioactive-crude compounds

The crude bioactive compound were spotted on the baseline of the silica gel plates (stationary phase) at 1 cm and then allowed to dry at room temperature.

The plates were placed in TLC chamber pre-saturated with the mobile phase as Toluene: Ethyl acetate: Methanol (solvent) in the ratio 1:0.8:0.2 and 1:0.5:0.5. The chromatogram was developed and visualized under UV light and in the iodine chamber, and then the spots were marked. The Rf values were calculated.

Rf value = Distance travelled by the solute / Distance travelled by the solvent.

In-vitro antioxidant activity of bioactive compounds

The bioactive components were separated from the ethyl acetate layer and the crude compound obtained was evaluated for assessing antioxidant property.

Phosphomolybdenum assay

Total antioxidant capacity can be calculated by the method described by.¹⁴ Various concentrations (150µg-450µg/ml) from the prepared sample was been pipetted out and 1ml of the reagent solution was added, followed by incubation in boiling water bath at 95°C for 90mins. After cooling the sample to room temperature, the absorbance of the solution was measured at 695 nm in UV spectrophotometer. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions.

Free radical scavenging activity

The antioxidant activity was determined by DPPH scavenging assay.¹⁵ Various concentrations (150µg-450µg/ml) of the crude extract was been pipetted out in clean test tubes. Freshly prepared DPPH (1,1-Diphenyl-2-picryl hydrazyl) solution (2ml) was added to each tube and the samples were incubated in dark at 37°C for 20 min and read at 517 nm. The data were expressed as the percent decrease in the absorbance compared to the control. The percentage inhibition of radical scavenging activity was calculated.

% of DPPH radical scavenging activity,

$$(\%RSA) = 100 * (\text{Abs. control} - \text{Abs. sample}) / \text{Abs. control}$$

Identification of bioactive metabolites by gas-chromatography mass spectrometry

The presence of active compounds were been confirmed by thin layer chromatography and the compounds were identified using gas chromatography and mass spectrometry (GC-MS) method, (TSQ QUANTUM XLS). The name of the instrument is Gas Chromatography-Mass Spectrometry and the instrument made is of Thermo scientific. The software required for analytical studies is XCALIBUR (ver-2.2). The column size is of TG-5MS (30mX0.25mmX0.25um). The injector temperature and interface temperature (°C) was at 280 °C.

RESULTS

Soil sample collection and pre-treatment

The soil samples were collected from Phytosphere regions of *Manilkara zapota* (sapota tree) and Muthukadu marine sediments from inner depth.

The collected samples were brought to laboratory in sterile bags, transferred to sterile petridishes and were allowed to drying process to avoid contamination and is depicted in the Figure 1a and Figure 1b.

Isolation and sub-culturing of Actinomycetes

After the incubation period of 7-10 days, clear white, powdery colonies were observed in 10⁻², 10⁻³, 10⁻⁴ dilutions of terrestrial soil and 10⁻¹, 10⁻² dilutions of marine soil (Table 1).

Table 1: Depicts the isolated colonies from respective dilutions by spread plate technique¹⁷

Sample	Terrestrial soil (TS)			Marine soil (MS)	
	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻¹	10 ⁻²
Isolates	TS	TS	TS	MS	MS
	1001	1005	1009	1001	1003
	TS	TS	TS	MS	MS
	1002	1006	1010	1002	1004
	TS	TS			MS
	1003	1007			1005
	TS	TS			
	1004	1008			

The incubated plates were observed after five days to check the appearance of the colonies. Whitish to pink pin-pointed colonies were observed and they were carefully isolated by streaking in sterile Starch casein agar plates. The isolated colonies from both marine and terrestrial soils were further purified by quadrant streak method (Figure 2).

Preliminary characterization of Actinomycetes isolates

Colony morphology

The colony morphology reveals with the colour of both aerial (front) and substrate (backside) mycelium, whether the isolate will be a pigment producer and also the appearance of the isolate when carefully observed (Table 2).

Light microscopy (coverslip culture technique)¹⁹

The colonies were found to have the characteristic morphology of actinomycetes.

Based on the microscopy studies, the potent isolates formed (Table 3) straight to flexuous (rectiflexibiles) spore chain on aerial mycelium with smooth surface (Figure 3a and Figure 3b), also other isolates formed Retinoculiaperti spore chain on aerial mycelium with smooth spore surface (Figure 3c) according to Shirling and Gottlieb.

Extraction of bioactive compounds from selected Actinomycetes

The secondary metabolites were produced in ISP-2 medium and specific strains producing various pigments such as yellow, brown, orange, pale green where been observed after the growth period (Figure 4).

Biochemical characterization²⁰

Gram staining

From the results of Gram staining, the selected isolates exhibited as gram positive, cocci in which it was observed as purple coloured cells under microscopic examination.

Table 2: Colony morphology of moderate and fast growing actinomycetes¹⁸

Isolate no.	Mycelium		Colony margin	Pigment production	Appearance
	Aerial	Substrate			
TS 1001	Pale yellow	Pale yellow	-	-	Mucous layered
TS 1003	Greyish white	Light orange	Serrated	Light orange (substrate)	Dry, powdery and fibrous
TS 1005	White	Pale yellow	Depression on top of colony	-	Greyish white, dry and powdery
TS 1006	Greyish yellow	Yellow			Dry and powdery
TS 1008	Pale green	Pale yellow			Light powdery and dry
TS 1009	White	Yellow			Chalky white and dry
TS 1010	Pale brown	Light brown			White colonies, powdery and dry
MS 1001	Greyish white	Dark yellow	Webbed colonies	Red (substrate) Pink (aerial and substrate)	Dry and ash like
MS 1003	Pale yellow	Cloudy grey			Dry, powdery and ash like
MS 1004	Pale yellow	Dark pink			Dry, ash like and powdery
MS 1005	Pale yellow	Reddish brown	Depression on top of colony	Reddish brown (substrate)	Ash like and chalky white

Diffusible pigment production test (glycerol asparagine agar medium)

The diffusible pigment production test was conducted for the strains which produced pigments and no diffusible pigments were observed in the Glycerol Asparagine agar medium (Table 4).

Melanin pigment production test (tyrosine agar medium)

The pigment producing strains showed positive results for melanin pigment production test except MS 1004 in Tyrosine agar medium. From Table 5 it is understood that certain isolates could produce melanin pigment which can induce many biological properties like antioxidant, antimicrobial activity.

Dot-blot assay for rapid radical scavenging activity for the crude extracts

The results of dot-blot assay showed coloured spots where the aliquots of crude extract of the selected isolates were placed in row. The purple zone on the plate indicates no (free radical scavenging) antioxidant activity and the yellow zone indicates antioxidant activity. The

more intense the yellow colour, the greater the antioxidant activity (Figure 5a and Figure 5b).

Table 3: Coverslip morphology-arrangement pattern.¹⁹

Isolates	Pattern
TS 1003	Rectus (R)
TS 1008	Monovorticillus Spira (MV-S)
TS 1009	Flexibilis (F)
TS 1010	Flexibilis (F)
MS 1001	Bivorticillus (BIV)
MS 1002	Rectus (R)
MS 1003	Bivorticillus (BIV)
MS 1004	Spira (S)
MS 1005	Spira (S)

Thin layer chromatographic analysis of crude compound

The TLC screening showed the presence of active compounds and among these TS 1010 showed better results (Figure 6) and its Rf values are given in Table 6. Thin layer chromatography was performed for the crude compound from the isolate TS 1010. This is mainly

performed regarding whether the compound can be gathered concerning the presence or absence of a metabolite. Natural products may be tracked by running analytical TLC of ethyl acetate fractions from other separation processes such as Column chromatography and HPLC. Effective visualization or deduction is an important criterion to obtain pure compounds. From the results obtained it is evident that the compound possesses

eight distinct clear bands under UV illuminator and four bands under iodine vapours.

When compared to TS 1010 the number of active compounds in isolates TS 1003, TS 1006, TS 1009 under UV illuminator were less effective (TS 1006-six bands, TS 1003 and TS 1009 - three bands).

Table 4: Diffusible pigment production test²⁰

ISP-5(glycerol asparagine agar medium)			
Isolate	Results	Isolate	Results
	Nil		Nil
TS 1005	-ve	MS 1003	-ve
	Nil		Nil
TS 1009	-ve	MS 1004	-ve
	Nil		Nil
MS 1001	-ve	MS 1005	-ve

Table 5: Melanin pigment production test²⁰

ISP-7 (tyrosine agar medium)			
Isolate	Results	Isolate	Results
	Pale yellow		Red
TS 1005	+ve	MS 1003	+ ve
	Light yellow		Nil
TS 1009	+ ve	MS 1004	- ve
	Pale brown		Orange
MS 1001	+ ve	MS 1005	+ ve

Table 6: Thin layer chromatography - Rf values of TS1010.

Solute front (cm)	Rf value
5.3	0.96
4.3	0.78
3.5	0.63
3.1	0.56
2.4	0.43
1.4	0.25
1.0	0.18
0.9	0.16

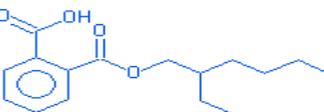
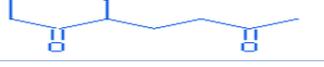
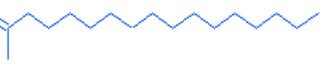
Solvent front = 5.5cm, Rf value = Solute front / Solvent front

Colony morphology of TS 1010

The isolate TS 1010 was studied for the aerial mycelium, substrate mycelium, colony margin, pigment production and appearance (Figure 7).

The aerial mycelium was found to be pale brown and substrate mycelium was found to be light brown. The colony margin belongs to depression on top of colony type. The isolate TS 1010 did not produce any pigment and the appearance was powdery in nature and dry with white colonies.

Table 7: Active compounds obtained from the gas-chromatogram analysis.

RT	IUPAC Name	Structure	Molecular weight (g/mol)	Molecular formula
17.02	Hexadecanoic acid, methyl ester		270.457	C ₁₇ H ₃₄ O ₂
15.57	1-octadecene		252.486	C ₁₈ H ₃₆
22.62	1,2-benzene dicarboxylic acid, mono(2-ethylhexyl) ester		278.34	C ₁₆ H ₂₂ O ₄
14.28	Cyclopenta decanone		224.388	C ₁₅ H ₂₈ O
17.68	9-Eicosene, (E)		280.54	C ₂₀ H ₄₀
10.9	Cyclohexanone, 2-(3-oxobutyl)		168.236	C ₁₀ H ₁₆ O ₂
13.28	1-tridecene		182.351	C ₁₃ H ₂₆
16.52	2-heptadecanone		254.458	C ₁₇ H ₃₄ O
18.78	10-octadecenoic acid, methyl ester		296.495	C ₁₉ H ₃₆ O ₂
19.58	Z,Z-3,15-octadecadien-1-ol acetate		308.499	C ₂₀ H ₃₆ O ₂
21.17	Cyclodocosane, ethyl		336.648	C ₂₄ H ₄₈
22.1	Tricosane-2,4-dione		352.603	C ₂₃ H ₄₄ O ₂

RT – Retention time

Table 8: Bioactivity of active compounds of ethyl acetate fraction of crude compound of TS1010.

RT	Active compounds	Bioactivity
17.02	Hexadecanoic acid, methyl ester	Antioxidant activity
15.57	1-octadecene	Antioxidant, antibacterial, anticancer activities
22.62	1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester	Cytotoxic activity, effective against HepG2, MCF 7 cell lines

***In-vitro* evaluation of antioxidant potential**

Phosphomolybdenum assay

The total antioxidant activity based on the reduction Mo (VI) to Mo (V) by the crude compound and formation of a Mo (V) complex at acidic pH increase in the absorbance value was well observed for the crude compound. The Phosphomolybdenum reducing potential was found to be 0.979 at the concentration of 450 µg/mL (Figure A) for the isolate TS 1003 and comparatively the reducing potential was found to be 0.528 for TS 1006 at the same concentration.



Figure 1(a) and (b): Soil sample before and after pre-treatment.

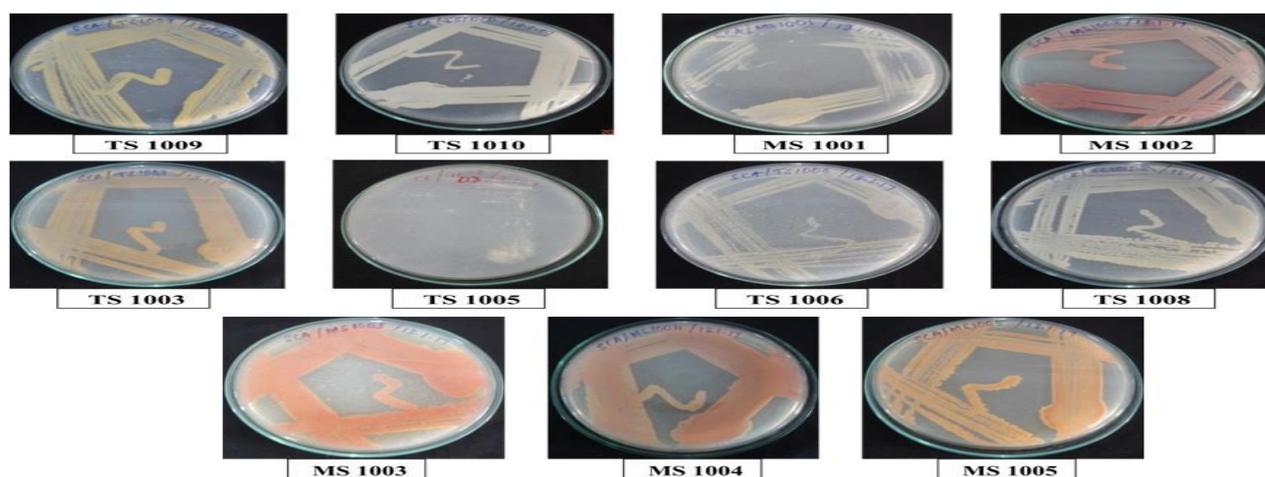


Figure 2: Sub-cultured actinomycetes strains-Quadrant streak method.

Free radical scavenging activity

The *Streptomyces* species isolated from the terrestrial sample collected from Phytosphere region were found to

have significant antioxidant activity. They have a similar mechanism in the absorption spectrum of the stable free radical changes when the molecule is reduced by an antioxidant or a free radical species. The isolate TS 1006

(Figure B) was found to be effective in scavenging the free radicals when compared to the isolate TS 1010 (Figure C). The antioxidant activity determined by DPPH assay had the maximum activity of 93.59% at the concentration of 650 µg/mL. The IC₅₀ for crude compound (TS 1006) was evaluated to be 334.56 µg/mL

at the concentration of 400 µg/mL. Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability.

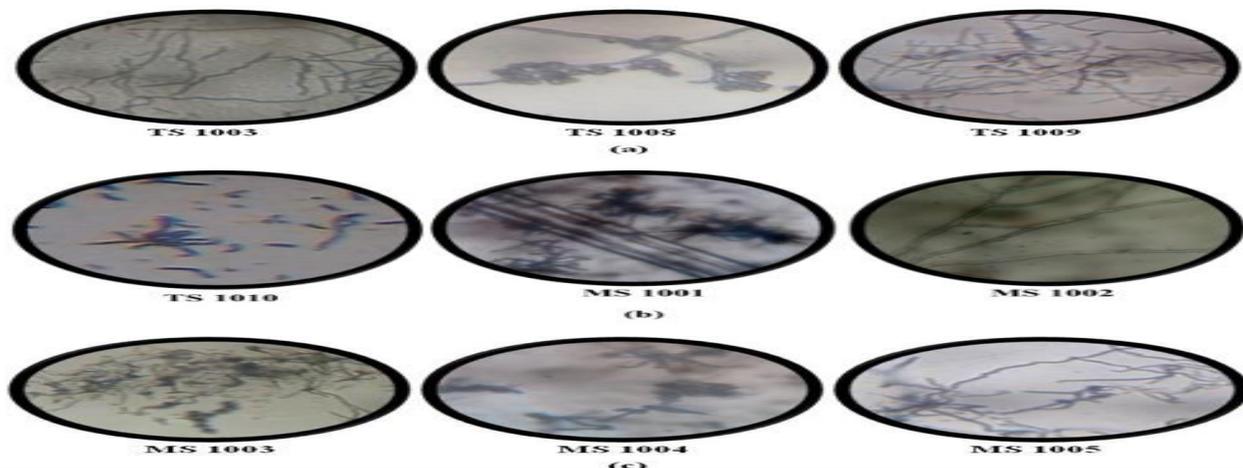


Figure 3 (a), (b) and (c): Microscopic view of the selected isolates by Coverslip culture method.

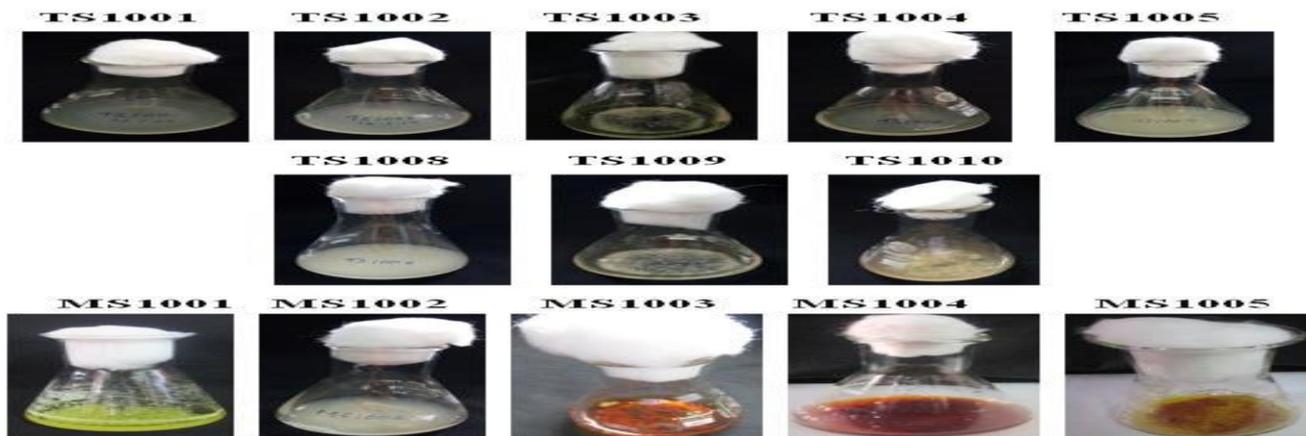


Figure 4: Small scale extraction of secondary metabolites.

Identification of bioactive metabolites by gas-chromatography mass spectrometry

Figure D shows the Gas Chromatogram of ethyl acetate fraction of crude compound (TS1010). The active compounds obtained from the Gas-Chromatogram analysis are given in Table 7.

From the Gas Chromatography Mass Spectrometry analysis three active compounds were found to have bioactivity (Table 8). Further, the specific compound

responsible for antioxidant activity can be evaluated for anti-proliferative activity to determine cytotoxicity effect.

DISCUSSION

15 different isolates were obtained from the collected samples; 10 isolates from terrestrial soil and 5 isolates from marine soil samples. They showed better and efficient growth in SCA medium (Starch Casein Agar). The isolated samples were further purified by sub culturing in the SCA medium by quadrant streak technique. The purified cultures were subjected to

preliminary characterization which included colony and surface morphology studies. The colony morphology showed the characteristic white, ash like, powdery nature of actinomycetes colony. The colony morphology of each isolate-its aerial and substrate mycelium colour, appearance of each colony namely; dew drop like, depression on top of colony were observed and noted. In the structural morphology characterization by Coverslip method; each coverslip inserted in SCA medium petriplates were streaked with each isolate and they were allowed to grow for a period of few days and then examined under a light microscope. The microscopic examination showed the morphological structures of each

isolate and was compared with those mentioned by Shirling and Gottlieb, 1966. The potent isolates were found to have straight to flexuous (rectiflexibiles) spore chain on aerial mycelium with smooth surface, also other isolates formed Retinoculiaperti spore chain on aerial mycelium with smooth spore surface. Based on the growth of isolates, colony and structural morphology, eleven isolates (TS 1001, TS 1003, TS 1005, TS 1006, TS 1008, TS 1009, TS 1010, MS 1001, MS 1003, MS 1004 and MS 1005) among the fifteen were selected and subjected to secondary metabolite extraction. They were grown in ISP-2 media for better and effective secondary metabolites production.

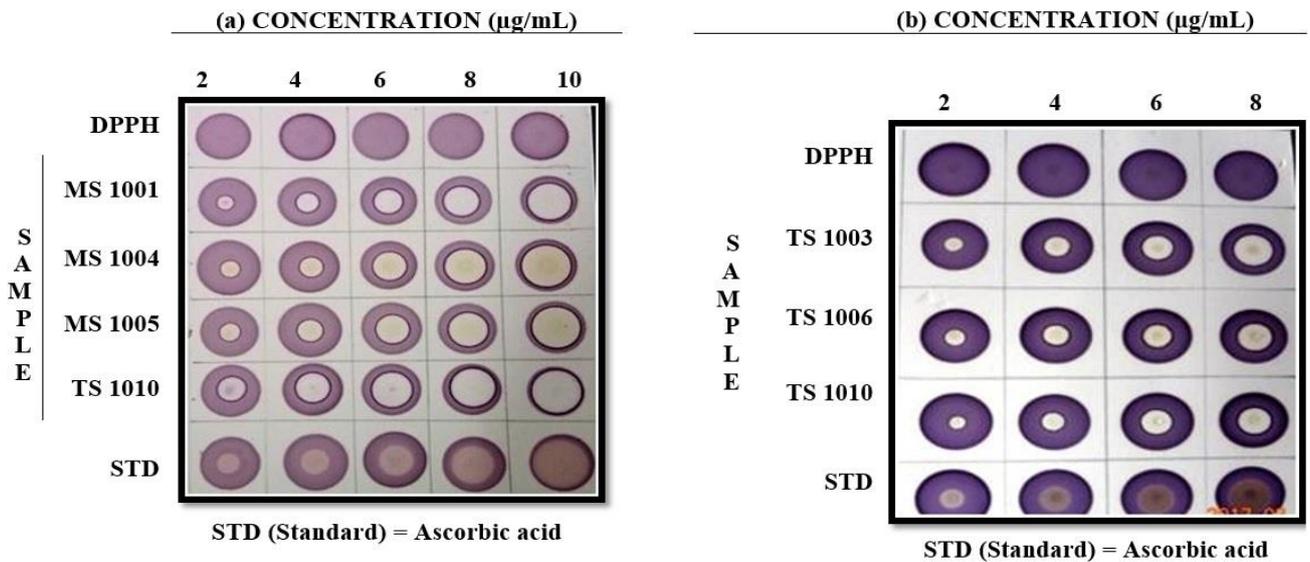


Figure 5 (a) and (b): Dot plot assay.

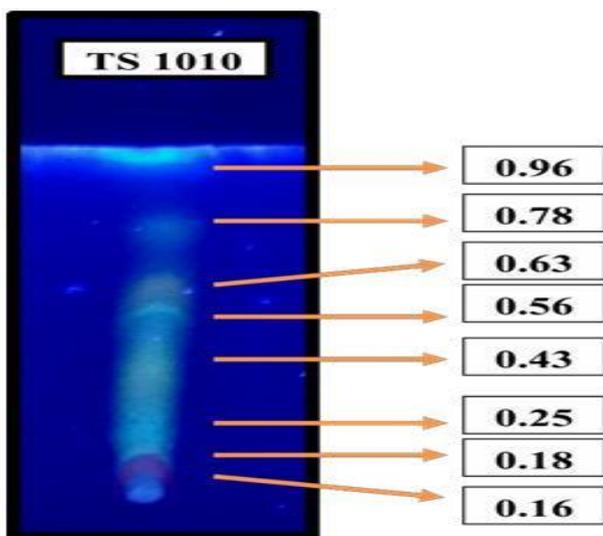


Figure 6: TLC screening of crude compound for the selected isolate.

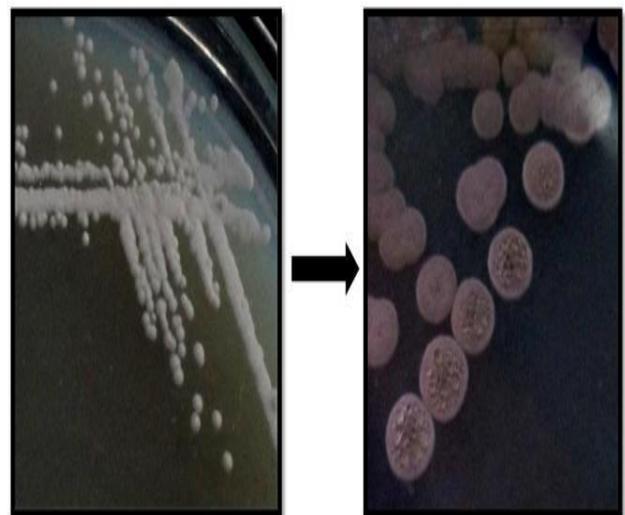


Figure 7: Colony morphology of TS1010.

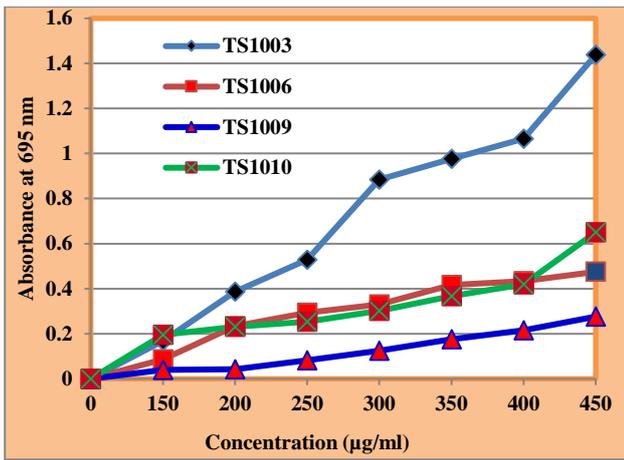


Figure 8: Total antioxidant activity for the selected isolates.

The eleven isolates were tested for gram positive bacteria by gram staining, under a light microscope some isolates showed cocci shaped bacteria with purple coloured stains indicating that they are gram positive bacteria (actinomycetes are gram positive bacteria). The small scale secondary metabolites production resulted in the production of pigments and hence the isolates TS 1005, TS 1009, MS 1001, MS 1003, MS 1004 and MS 1005 were tested for pigment production by diffusible pigment production and Melanin pigment production tests using ISP-5/Glycerol Asparagine agar and ISP-7/Tyrosine agar media respectively. They test showed nil diffusible pigment production in Glycerol Asparagine Agar medium and positive results (yellow or orange to brown coloured pigments) in Tyrosine agar medium indicating the production of melanin pigments. The secondary metabolites of isolates produced by small scale were extracted and tested for the presence of active compounds and their radical scavenging activity by TLC and from Dot plot assay it was observed that TS 1003, TS 1006, TS 1009 and TS 1010 showed better radical scavenging activity.

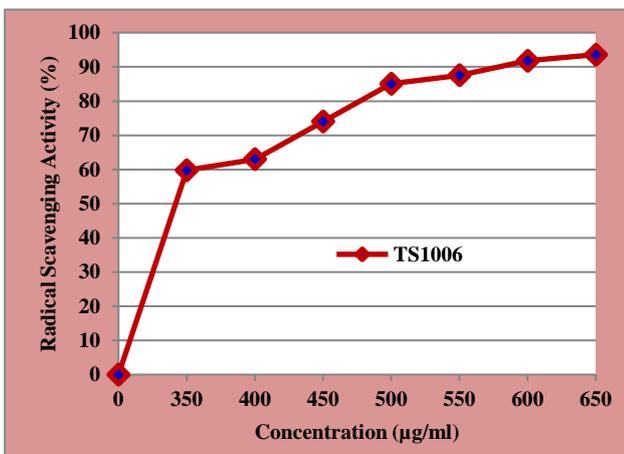


Figure 9: Radical scavenging activity by DPPH method for TS1006.

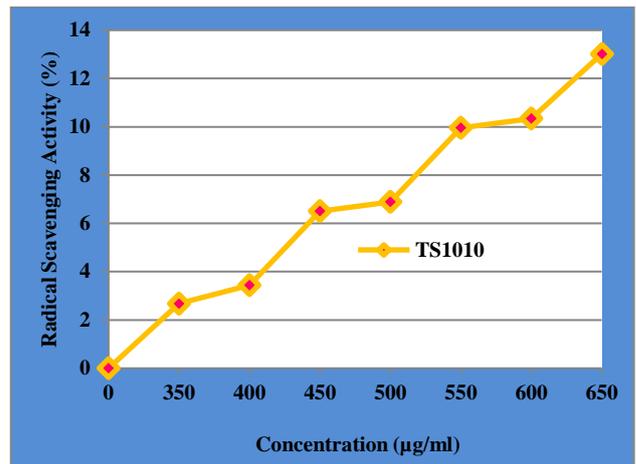


Figure 10: Radical scavenging activity by DPPH method for TS1010.

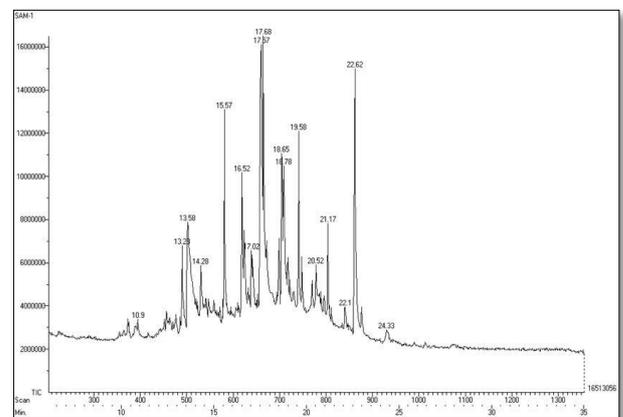


Figure 11: Gas chromatogram of ethyl acetate fraction of crude compound (TS1010).

Based on the above characterizations and studies, four isolates (TS 1003, TS 1006, TS 1009 and TS 1010) were selected for bulk production in ISP-2 media. The secondary metabolites were extracted after a period of 8-10 days by filtration, centrifugation, and condensation of the supernatant to obtain the crude extracts. The crude extracts of the following four isolates namely - TS 1003, TS 1006, TS 1009 and TS 1010 were screened for the presence of bioactive compounds by TLC. When viewed under a UV illuminator, the isolates TS 1010 and TS 1006 were observed to possess more active compounds than TS 1003 and TS 1009 both in UV illuminator and in iodine. The antioxidant potential of crude extracts of the four isolates determined by DPPH assay and Phosphomolybdenum assay resulted in the selection of the isolate TS 1010 for further studies as it showed better radical scavenging activity by Dot plot assay as well. The crude compound of the isolate TS 1010 was analyzed by GC-MS to obtain the information regarding the bioactive compounds that were observed under UV illuminator by TLC. GC-MS showed a list of thirteen bioactive

compounds and among them three were found to have antimicrobial, anticancer, antibacterial activities.

CONCLUSION

Streptomyces sp. showed various bioactive properties, which highlighted its importance as potential pharmacological agents. Hence, there could be probability of new bioactive compound in the crude extract, which might provide a basis for further development of novel compound from *Streptomyces* sp. This also provided a new insight towards the development of good candidates for pharmaceutical and bioactive natural products. The development of drugs can be turned as a candidate for curing several types of diseases.

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